Role of Melatonin in Monochromatic Light Affecting the Expression of Somatostatin in Hypothalamus and Pituitary of Chicks

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Research

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Abstract

Background: Melatonin (MEL) plays an important role in regulating the growth and development of organism and the metabolism of cells. A study was conducted to explore the effect of MEL-mediated monochromatic light on the secretion of somatostatin (SST) in the hypothalamus and pituitary.

Results: The newly hatched broilers were exposed to white (WL), red (RL), green (GL) and blue (BL) lights through the pinealectomy model. The results showed that SST immunoreactive neurons and fibers were distributed in hypothalamus. The mRNA and protein levels of SST and SST receptor 2 (SSTR2) in hypothalamus and pituitary in RL were higher than those of in GL and BL. After pinealectomy, mRNA and protein levels of SST and SSTR2 in hypothalamus and pituitary in different light groups were increased and the differences were disapeared. Meanwhile, the trend of *SSTR5* mRNA in pituitary is the same as the *SSTR2* mRNA in pituitary. In vitro, the exogenous SST inhibited the growth hormone (GH) secretion, and SSTR2 and SSTR5 selective antogonists promoted GH secretion. While, melatonin receptor 1b (Mel1b) and Mel1c selective antagonists increased relative concentraitons of SST in adenohypophysis cells.

Conclusion: Monochromatic light affected the expression of SST in chick hypothalamus and pituitary. MEL via Mel1b and Mel1c decreased SST secretion under GL, which is related to inhibiting the combination of SST, SSTR2 and SSTR5 in adenohypophysis cells.

Introduction

The growth and development of birds are influenced by the light information such as light schedule, intensity and wavelength. Light is transmitted to the visual center through the retinal ganglion cells [1] or acted on the photoreceptors in the brain through the skull [2]. Then light information is converted into the biological signals, which affects the secretion of plasma hormones through the pituitary portal system and regulates the physiological function of the body [3]. Previous studies have found that green light significantly elevated liver insulin growth factor-1 (IGF-1) secretion [4], skeletal satellite cells proliferation [5, 6], muscle growth [7, 8], and improved meat quality in broilers [9, 10]. While, the growth hormone [11] plays an important role in promoting the growth and development and improving the productive performance of broilers [12].

GH is one of the main regulatory hormones for avian growth, which is regulated by growth hormone releasing hormone (GHRH) and somatostatin (SST)[13, 14]. SST plays a biological role combined with the five different subtypes of the somatostatin receptors (SSTRs)[14]. Studies have shown that GHRH immunoreactive neurons are distributed in the infundibular nucleus around the third ventricle, and green light improves GHRH mRNA and protein levels in hypothalamus and promotes the plasma GH secretion in the early stage of broilers [15]. However, chicken SST was able to inhibit the basal GH release and GHRH-stimulated GH secretion [16], and affected the secretion of GH by dose-dependent and receptor-specific patter [17]. In addition, SST expressed in chick pituitary was different from the mammals [18] and primates [17]. However, it is unclear whether the monochromatic light affects SST levels in the

hypothalamus and pituitary, and which types of SSTR affect GH secretion under monochromatic light in chick.

Melatonin [19] is ubiquitous in animals from the single-cell organisms to the higher vertebrates, which plays an important role in regulating the growth and development of organism [19] and the metabolism of cells [20]. Previous studies showed that plasma MEL is positive correlated with plasma GH and hypothalamic GHRH proteins; the levels of plasma GH, mRNA and protein of hypothalamic GHRH decrease after pinealectomy [15, 21]; and MEL mediates the monochromatic green light to affect the expression of pituitary-specific transcription factor-1 to increase plasma GH through melatonin receptor 1b (Mel1b) and Mel1c [21]. However, whether MEL mediates the monochromatic light to affect SST levels in hypothalamus and pituitary, which subtypes of MEL receptors affect pituitary SST secretion, and what intracellular signal transduction in chick need to be further explored.

Materials And Methods

Animal treatments and sampling

One hundred and forty-four newly hatched Arbor Acre male broilers (post-hatching day 0, P0) from Beijing Huadu Breeding Corporation were randomly divided into four groups, which were reared under the different LED lamps, white (WL, 400–700 nm), red (RL, 626 nm), green (GL, 514 nm) and blue light (BL, 466 nm) until P14. The light intensity was 0.025 W/m^2 at the bird-head level, and schedule was 23 h daily (L:D = 23 h:1 h, lights off at 2300). Feed and water *ad libitum*. The sham operation (Sham) and pinealectomy (PINX) were performed at P3 according to the previous study [4]. Each light group included intact (n = 10), Sham (n = 10) and PINX (n = 10) treatments. The remaining 24 birds were reared under GL for in vitro test.

At P14, 120 birds from each light treatment were killed by exsanguination under anesthesia with Nembutal (30–40 μg/g). The hypothalamus and anterior pituitary were removed and frozen in liquid nitrogen for quantitative reverse transcription polymerase chain reaction (RT-qPCR) and western blot. The hypothalamus from the intact white light group of three birds were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer solution (PBS, pH 7.4) for 48 h, and performed the frozen (40 μm in thickness) and paraffin sections (10 μm in thickness) for immunohistochemical staining.

Immunohistochemical staining

The sections were incubated overnight at 4℃ with SST polyclonal antibodies (Enzo Life Scienes, USA). Then sections were rinsed in 0.01 M PBS and incubated with a biotinylated goat anti-rabbit IgG (Cowin Bio., China) for 3 h at room temperature. After washing, the sections were incubated with streptavidin-conjugated horseradish peroxidase (Vector Laboratories, USA) for 2.5 h at room temperature. The immunoreactivity was visualized by incubating in 0.01 M PBS containing 0.05% 3’,3-diaminobenzidine tetrahydrochloride (DAB; Sigma, USA) and 0.003% hydrogen peroxide for 15 min in the dark. The sections were then mounted, stained with hematoxylin, and the control slides without primary antibodies were

examined (data not shown). The immunoreactive cells exhibited yellow-brown staining in the cytoplasm of the perikarya. The immunoreactive neurons and fibers were observed by an Olympus BX51 microscope (Japan) according to the stereotaxic atlas ofchick brain (Kuenzel and Masson, 1988).

RT-qPCR

The protocol of RNA extraction and RT-qPCR amplification inhypothalamus and pituitary were referenced to the previous study [21]. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal standard, and the $2^{-\triangle\triangle Ct}$ method was used to analyze each sample and gene. The PCR primers are listed in Table 1.

Table 1
Sequences of the primers for RT-qPCR.

Genes	Primer sequences (5'-3')	Produce size (bp)	Accession No.
SST	F: TCTGGAGTCGGAGGACTTGT	124	NM_205336.1
	R: GAAGTTCTTGCAGCCCGCTT		
SSTR2	F: TCTCCATCGTGGTTGCTGTC	113	NM_001030345.2
	R: GTCGAACATGCCCTTGAGGA		
SSTR5	F: TCGTGTTGGGTCTACAAGGC	275	NM_001024834.1
	R: CCTTTCGGAGGCAAAGGACT		
GAPDH	F: ATCACAGCCACACAGAAGACG	125	NM_204305.1
	R: TGACTTTCCCCACAGCCTTA		

Western blot

The protocol of protein extraction and protein concentration examination in hypothalamus and anterior pituitary were according to the previous study [21]. The protein concentration was adjusted to 2 mg/mL. Each sample of 100 μg of protein was separated to 12% SDS-PAGE, and then it was electrotransferred into apolyvinylidene difluoride membrane (Millipore, USA). After blocking with 5% skim milk/Tris-buffered saline and Tween-20 (TBST) for 2 h at room temperature, the blots were probed with SST polyclonal antibodies (Enzo Life Scienes, USA), SSTR2 (MyBioSource, USA) or β-actinantibody (Proteintech, China) overnight at 4℃. After washing with TBST for three times, the membranes were exposed to horseradish peroxidase-conjugated goat anti-rabbitIgG (Cowin Bio.) for SST and SSTR2, or goat anti-mouse IgG (Cowin Bio.) for β-actin for 2 h at room temperature. The immunoblots were visualized with anelectrochemiluminescence system (Millipore, USA) and were analyzed using image analysis software (ImageJ, USA). The results were expressed as the integral optical density (IOD) of the target band versus the IOD of the β-actin bands, which were tested in triplicate.

Isolation and primary culture of adenohypophysis cells

According to the previouse study [21], the adenohypophysis cells from 24 anterior pituitaries of birds were isolated, and cultured in an incubator at 37°C and 5% CO_2 for 48 h. Then, a part of adenohypophysis cells were incubated in 0.001% ethanol (as vehicle), 1.0×10^{-7} M SST (Phoenix Biotech Co., China), 1.0×10^{-6} μM CYN 154806 (SSTR2 antagonist; Abcam, USA), or 1.0×10^{-7} M BIM 23056 (SSTR5 antagonist; Abcam), respectively. Another part of adenohypophysis cells were incubated in 0.001% ethanol (as vehicle), 1.0×10^{-7} M luzindole (nonselective Mel1a/Mel1b antagonist; Santa Cruz Biotechnology Inc., USA), 1.0×10^{-8} M 4-phenyl-2-propionamidotetralin (4P-PDOT, selective Mel1b antagonist; Tocris Bioscience, UK) or 1.0×10^{-7} M prazosin (selective Mel1c antagonist; Santa Cruz Biotechnology Inc.) for 30 min, followed by the addition of melatonin (dissolved inethanol 400 pg/mL; Sigma), respectively. The cells were cultured in an incubator at 37°C and 5% CO_2 for 24 h. The control was incubated with serum-free Dulbecco's modified Eagle medium (DMEM; Gibco, USA).

After 24 h, the suspension of adenohypophysis and the adenohypophysis cells were collected separately, and the experiment was divided into two parts. One was used to measure SST (Meimian Industrial Co., China) and GH (Meimian Industrial Co.) concentrations in the suspension of adenohypophysis by enzyme-linked immunosorbent assay (ELISA) kits for chick after centrifuging (5000 g, 10 min,4 °C). The optical density (OD) was measured by an automated ELISA reader (Bio-Rad, USA) at 450 nm. The another was used to determine mRNA levels of *SST* by RT-qPCR.

Data analysis

To normalize values within each group, the values obtained were compared with WL or cell control (set at 1). Each sample was tested in triplicate. Three to six chicks from each treatment group were measured in vivo. The data were expressed as the mean ± standard error (mean ± SEM). The differences in lights and treatments were analyzed with two-way analysis of variance (ANOVA), and the differences in the exogenous addition were analyzed with one-way ANOVA using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered significant difference. All of the graphs were plotted using GraphPad Prism 7.0 (GraphPad Software, Inc.).

Results

Distribution of SST immunoreactive neurons and fibers in hypothalamus

The results of immunohistochemical staining in the hypothalamus of chick showed that SST immunoreactive neurons were distributed in the paraventricular nucleus (PVN), ventromedial nucleus (VMN), dorsal supraoptic decussation (DSD) and infundibular nucleus (IN). The shape of SST immunoreactive neurons were fusiform, round or oval. The cytoplasm was dark brown, the nucleus was obvious, and some neurons streched out long processes. Meanwhile, SST immunoreactive fibers were distributed in the median eminence (ME), as well as in PVN, VMN, DSD and IN (Fig. 1).

Effects of monochromatic lights and different treatments on the levels of *SST* mRNA in hypothalamus and pituitary

There was no interaction between monochromatic lights and different treatment groups on the levels of *SST* mRNA in hypothalamus ($F_{6,52}$ = 0.505, P = 0.801) and pituitary ($F_{6,48}$ = 0.491, P = 0.812). The results showed that monochromatic lights and treatment groups significantly affected *SST* mRNA levels in hypothalamus (monochromatic lights: $F_{3,52}$ = 3.459, P = 0.023; treatments:$F_{2,52}$ = 17.705, P = 0.000) and pituitary (monochromatic lights: $F_{3,48}$ = 5.456, P = 0.003; treatments: $F_{2,48}$ = 56.509, P = 0.000). The main effect analysis showed that *SST* mRNA in hypothalamus and pituitary in RL were higher than those of in GL by 25.40% (P = 0.025) and 13.60% (P = 0.001). However, no difference was observed between other monochromatic lights. Meanwhile, *SST* mRNA in hypothalamus and pituitary in pinealectomy group were higher by 37.45%-39.88% (hypothalamus, P = 0.000) and 26.46%-27.23% (pituitary, P = 0.000) than those of in intact and sham operation groups, respectively (Fig. 2A and 2B).

Effects of monochromatic lights and different treatments on the levels of SST protein in hypothalamus and pituitary

There was no interaction between monochromatic lights and different treatment groups on the levels of SST protein in hypothalamus ($F_{6,45}$ = 0.727, P = 0.630). The results showed that the levels of SST protein in hypothalamus were significantly affected by monochromatic lights ($F_{3,45}$ = 9.516, P = 0.000) and treatment groups ($F_{2,45}$ = 15.859, P = 0.000). The main effect analysis showed that hypothalamic SST protein in RL were higher than those of in GL by 16.37% (P = 0.000) and in BL by 14.17% (P = 0.001), respectively. However, there was no significant difference between GL and BL (P > 0.05). Meanwhile, SST protein in the pinealectomy group was higher by 12.54% (P = 0.000) and 15.58% (P = 0.000) than that of in intact and sham operation groups, respectively (Fig. 2C).

There was interaction between monochromatic lights and different treatment groups on the levels of SST protein in pituitary ($F_{6,45}$ = 8.021, P = 0.000). The interaction analysis showed that the SST protein levels in the intact and the sham operation groups in GL and BL was lower by 20.43%-23.22% and 16.37%-19.28% than that of in RL (P = 0.000), respectively. However, the SST protein levels in the pinealectomy group in BL was higher by 1.38% than that of in WL (P > 0.05). The difference was 16.80% (P = 0.000) (Fig. 2D).

Effects of monochromatic lights and different treatments on the levels of *SSTR2* and *SSTR5* mRNA in hypothalamus and pituitary

There was no interaction between monochromatic lights and different treatment groups on the levels of *SSTR2* mRNA in hypothalamus ($F_{6,54}$ = 0.879, P = 0.516) and pituitary ($F_{6,48}$ = 1.699, P = 0.142). The levels of *SSTR2* mRNA in hypothalamus and pituitary were significantly influenced by monochromatic lights (hypothalamus: $F_{3,54}$ = 7.515, P = 0.000; pituitary: $F_{3,48}$ = 6.425, P = 0.001) and treatment groups (hypothalamus: $F_{2,54}$ = 31.272, P = 0.000; pituitary: $F_{2,48}$ = 62.088, P = 0.000).The main effect analysis showed that *SSTR2* mRNA in RL was higher than that of in GL and BL by 19.46% (P = 0.001) and 17.01% (P = 0.002) in hypothalamus, and by 15.37% (P = 0.002) and 14.59% (P = 0.005) in pituitary, respectively. Meanwhile, *SSTR2* mRNA in pinealectomy group was higher than intact and sham operation groups by 28.15% (P = 0.000) and 26.86% (P = 0.000) in hypothalamus, and by 37.38% (P = 0.000) and 31.07% (P = 0.000) in pituitary, respectively (Fig. 3A and 3B).

There was no interaction between monochromatic lights and different treatment groups on the levels of *SSTR5* mRNA in the hypothalamus ($F_{6,45}$ = 0.121, P = 0.993) and pituitary ($F_{6,45}$ = 2.010, P = 0.084). The statistical analysis showed that the treatment groups significantly affected the levels of *SST5* mRNA in hypothalamus ($F_{2,45}$ = 3.517, P = 0.038) and pituitary ($F_{2,45}$ = 56.708, P = 0.000). The main effect analysis showed that *SSTR5* mRNA in pinealectomy group in hypothalamus was lower by 21.91% than that of in intact group (P = 0.043). However, the unweighted marginal mean value of *SSTR5* mRNA in pinealectomy groupin pituitary was higher by 47.83% than that of in intact group (P = 0.000) (Fig. 3C and 3D).

Effects of monochromatic lights and different treatment groups on the levels of SSTR2 protein in hypothalamus and pituitary

There was no interaction between monochromatic lights and different treatment groups on the levels of SSTR2 protein in hypothalamus ($F_{6,43}$ = 1.868, P = 0.108) and pituitary ($F_{6,45}$ = 1.775, P = 0.126). However, the levels of SSTR2 protein in hypothalamus and pituitary were influenced by monochromatic lights (hypothalamus: $F_{3,43}$ = 12.667, P = 0.000; pituitary: $F_{3,45}$ = 10.250, P = 0.000) and treatment groups (hypothalamus: $F_{2,43}$ = 85.650, P = 0.000; pituitary: $F_{2,45}$ = 102.038, P = 0.000). The main effect analysis results showed that SSTR2 protein in hypothalamus and pituitary in RL were higher than those of in the other light groups by 11.63%-21.74% (P = 0.000-0.013) and 5.16%-9.62% (P = 0.000-0.038), respectively. Meanwhile, SSTR2 protein of hypothalamus and pituitary in pinealectomy group were higher by 36.73%-42.64% (P = 0.000) and 20.13%-20.34% (P = 0.000) than those of other treatment groups, respectively (Fig. 3E and 3F).

Effects of CYN 154806 and BIM 23056 on GH secretion of adenohypophysis cells

The exogenous SST can significantly inhibit GH secretion in adenohypophysis cells, and the relative concentration of GH was lower by 26.30%-26.43% than that of the control (P = 0.000) and vehicle group

(P = 0.000). When CYN 154806 (SSTR2 antagonist) and BIM 23056 (SSTR5 antagonist) were added separately, the GH secretion in adenohypophysis cells were higher by 16.74%-18.49% than that of control (P = 0.000-0.001) and vehicle groups (P = 0.000-0.001). When SST is combined with CYN 154806 and BIM 23056, respectively, GH levels in the supernatant of adenohypophysis cells were significantly lower than that of CYN 154806 and BIM 23056 groups (18.45%-24.08%, P = 0.000), and was higher than that of SST alone (22.07%-29.40%, P = 0.000-0.003; Fig. 4).

Melatonin receptors mediated the expression and secretion of SST in adenohypophysis cells

MEL and melatonin receptors (Mel1a, Mel1b and Mel1c) antagonists affected *SST* mRNA levels ($F_{10,33}$ = 22.002, P = 0.000) and SST concentraitons ($F_{10,55}$ = 23.961, P = 0.000) in chick adenohypophysis cells (Fig. 5). When melatonin receptor antagonist was added alone, the synthesis and secretion of SST were not affected (P > 0.05). Compared with the control group, the level of *SST* mRNA and the concentration of SST after MEL added were decreased by 20.78% (P = 0.000) and 10.33% (P = 0.000), respectively. Compared with MEL group, the levels of *SST* mRNA and concentration of SST increased by 37.68% (P = 0.000) and 14.95% (P = 0.000) in MEL + 4P-PDOT, 36.26% (P = 0.000) and 15.11% (P = 0.000) in MEL + Prazosin, 53.75% (P = 0.000) and 18.08% (P = 0.000) in MEL + Luzindole + Prazosin, and 69.41% (P = 0.000) and 29.09% (P = 0.000) in MEL + 4P-PDOT + Prazosin, respectively. However, there was no difference in *SST* mRNA and SST concentration between MEL + Luzindole and MEL groups (P > 0.05).

Discussion

Previous study showed that the level of SST in hypothalamus is the highest in chick brain [22]. During the chick embryo development, the levels of SST in hypothalamus were kept at a low level from embryonic day 14 (E14) to E17. At E18, the level of SST in the hypothalamus was twice than that of E17, and reached a peak at the time of hatching day, and then the concentration of SST gradually decreased [11]. Using radioimmunoassay to detect the SST concentration at one-day-old male layer chicks, Geris et al. [22] found that SST were expressed in hypothalamic ME, DMN, VMN, PHN, AM, POP, POM, PVN and nCPa, and SST concentration in ME was the highest. However, the SST positive neurons and fibers were only detected in PVN, DSD, VMN, IN and ME at P14 by immunohistochemistry in this study (Fig. 1). The reasons for the different results may be related to the breed, age, and the detected methods.

SST is relatively conservative in the evolution of vertebrates. For example, SST-14 in frog is an effective inhibitor of GHRH-stimulated GH secretion [23]; SST-14 and SST-28 in goldfish can inhibit GH secretion in adenohypophysis [24]; SST in amphibian and reptile can inhibit thyrotropin releasing hormone stimulated release of GH from pituitary [25]; while SST in chicken can inhibit the basal GH release and GHRH-stimulated GH secretion [16]. Different from mammal, SST were expressed in the hypothalamus and pituitary of chick [22]. In order to investigate the mechanism of effect of monochromatic light on the growth and development of chick, the levels of SST mRNA and protein in hypothalamus and pituitary were detected by RT-qPCR and Western blot. The results showed that color of lights affected the levels of

SST in hypothalamus and pituitary of chick, which were the highest in RL and the lowest in GL (Fig. 2). The results are consistent with Zhang et al. [15] and Yue et al. [21]. GL promotes the expression of GHRH in hypothalamus and secretion of GH in plasma, while RL is the opposite. Therefore, SST is an important regulator of GH secretion, and GHRH and SST in the hypothalamus, as well as SST and GH in the pituitary play an important role in the growth and development of chick under monochromatic light. However, the biological function of SST mediated through five different subtypes of SSTRs, namely SSTR1-5. Meng et al. [14] detected the expression of SSTRs in different tissues of the adult Roman chick by PCR, and found that the expression levels of SSTR2 in hypothalamus, and SSTR2 and SSTR5 in pituitary were higher than others. Similarly, the changes of SSTR2 in hypothalamus, SSTR2 and SSTR5 in pituitary under the different light groups were similar to SST in hypothalamus and pituitary (Fig. 3). It has been reported that SSTR2 selective agonist strongly inhibited both the basal and the GHRH-stimulated GH release at low nanomolar concentrations, while SSTR5 selective agonist inhibited GH release only under basal conditions [26]. In order to verify the results of in vivo experiments, the adenohypophysis cells were cultured and supplemented the exogenous antagonists of SSTR2 and SSTR5 to observe the effect of SST on GH secretion in adenohypophysis cells under the monochromatic green light. The results showed that the exogenous addition of SSTR2 antagonist (CYN154806) and SSTR5 antagonist (BIM23056) could alleviate the inhibited effect of SST on GH secretion in adenohypophysis cells. And when SST was combined with the receptor antagonists of SSTR2 and SSTR5 respectively, the inhibited effect of SST on GH secretion in adenohypophysis cells decreased (Fig. 4). It can be concluded that the inhibition of GH secretion by SST is achieved by SSTR2 and SSTR5.

MEL is a multi-effect hormone secreted by pineal gland, which can widely regulate the physiological function of the organism [27]. Previous studies found that monochromatic light influence the level of MEL in plasma [28, 29], which is related to the growth and development in chick [4]. Therefore, pinealectomy was used to reduce the level of MEL in the circulating blood and explore the role of MEL in effect of monochromatic light on the secretion of SST in hypothalamus and pituitary. The results showed that the levels of SST in hypothalamus and pituitary increased after pinealectomy (Fig. 2), suggesting that MEL mediated the expression of SST in hypothalamus and pituitary of chick under monochromatic light. This result is consistent with the decrease of *GH* mRNA in pituitary and GH in plasma after pinealectomy [21]. Therefore, the above results illustrated that pineal gland MEL inhibited the synthesis and secretion of SST in hypothalamus and pituitary, thus weakened the inhibition of SST on the synthesis and secretion of GH in adenohypophysis, increased the level of GH in plasma, and promoted the growth. Meanwhile, after pinealectomy, the levels of SSTR2 in hypothalamus and SSTR2 and SSTR5 in pituitary increased in all light groups, and the differences between various light groups disappeared (Fig. 3). These demonstrated that MEL specifically affects the expression of SSTR in hypothalamus and pituitary.

Some reports showed that MEL can regulate the physiological function of organism through different types of receptors on the target cells, and it has tissue specificity in chick. For example, Mel1a and Mel1c mediate monochromatic light-induced the proliferation of B lymphocytes in bursa of Fabricius [30]. Mel1b and Mel1c mediate monochromatic light-induced the proliferation of T lymphocytes in chick thymus [31],

and the secretion of GH in adenohypophysis cells [21]. Mel1c mediates monochromatic light-induced the secretion of IGF-1 in chick liver [4]. Meanwhile, Mel1c inhibited GnRH expression via GnIH neurons in chick hypothalamus [32]. Whether MEL receptors mediated the secretion of SST is the same as those of MEL receptors mediated GH secretion in adenohypophysis under monochromatic green light? We found that the exogenous addition of MEL decreased the SST secretion in adenohypophysis cells (Fig. 5). When 4P-PDOT (Mel1b selective antagonist) or prazosin (Mel1c selective antagonist) were added, the secretion of SST in adenohypophysis cells increased. When 4P-PDOT and prazosin were added together, the secretion of SST in adenohypophysis cells increased more significantly. The above results indicate that Mel1b and Mel1c mediate the secretion of SST in chick adenohypophysis cells. Combined with our previous studies [21], it is demonstrated that MEL have direct and indirect dual effects on GH secretion in adenohypophysis cells, that is, MEL can directly promote the secretion of GH in adenohypophysis cells through Mel1b and Mel1c, and it can also indirectly promote the secretion of GH by inhibiting SST in hypothalamus and pituitary under GL.

Abbreviations

MEL: Melatonin; SST: Somatostatin; WL: White light; RL: Red light; GL: Green light; BL: Blue light; SSTR: Somatostatin receptor; GH: Growth hormone; Mel1b: Melatonin receptor 1b; GHRH: Growth hormone releasing hormone; IGF-1: Insulin growth factor-1; 4P-PDOT: 4-phenyl-2-propionamidotetralin; PVN: Paraventricular nucleus; VMN: Ventromedial nucleus; DSD: Dorsal supraoptic decussation; IN: Infundibular nucleus; ME: Median eminence.

Declarations

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All animal procedures were approved by the Animal Welfare Committee of the Agricultural Research Organization, China Agricultural University (Beijing).

Competing interests

All authors declare that they have no competing interests.

Consent for publication

Not applicable.

Authors' contributions

JC, XJQ and XFL conceived the study and designed the experiment, and prepared the draft of the manuscript; XJQ, XFL, XYY and MZL performed the experiments, including immunohistochemical analysis and analyzed the experimental data. ZXW and YLD provided comments and technical support. ZXW and YXC participated in its design and helped to revise the manuscript. The authors read and approved the final version of this manuscript.

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